

Amendments

In the Specification:

Please replace paragraph [0018], appearing at page 5, with the following amended paragraph:

[0018] Fig. 7 shows the presence of the 17 kDa subunit of caspase 3 in PANC-1 cells following treatment with a combination of the TfRscFv-liposome A-antisense HER-2 complex and gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ in comparison to untreated cells and to treatment with either gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ alone, the TfRscFv-liposome A-AS HER-2 complex alone, or the combination of TfRscFv-liposome A-scrambled HER-2 complex and gemcitabine HCl (GEMZAR®) ~~Gemzar®~~.

Please replace paragraph [0019], appearing at page 6, with the following amended paragraph:

[0019] Fig. 8 shows the presence of the 17 kDa subunit of caspase 3 in plasma from mice bearing PANC-1 xenograft tumor following i.v. administration of a combination of a complex of TfRscFv-liposome A-antisense HER-2 and gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ in comparison to an untreated animal or to treatment with either gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ alone, the TfRscFv-liposome A-AS HER-2 complex alone or the combination of a complex of TfRscFv-liposome A-scrambled HER-2 and gemcitabine HCl (GEMZAR®) ~~Gemzar®~~.

Please replace paragraph [0020], appearing at page 6, with the following amended paragraph:

[0020] Fig. 9A and 9B show *in vitro* down-modulation of protein expression in apoptotic pathways by TfRscFv-liposome A-antisense HER-2 alone or in combination with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ eight hours post-transfection of PANC-1 and COLO357 cells, respectively. Both types of cells showed clear evidence of the presence of the 17 kDa subunit of caspase 3. These results are contrasted to the results in untreated cells

and in cells which were treated with either gemcitabine HCl (GEMZAR®) Gemzar® alone or a combination of gemcitabine HCl (GEMZAR®) Gemzar® and TfRsvFv-liposome A-scrambled HER-2.

Please replace paragraph [0021], appearing at page 6, with the following amended paragraph:

[0021] Fig. 10 shows *in vitro* down-modulation of protein expression in apoptotic pathways by TfRscFv-liposome A-antisense HER-2 alone or in combination with gemcitabine HCl (GEMZAR®) Gemzar® sixteen hours post-transfection of PANC-1 cells. Controls as in Figures 9A and 9B.

Please replace paragraph [0022], appearing at page 6, with the following amended paragraph:

[0022] Fig. 11 shows the localization of the antisense HER-2 effect in tumor cells following i.v. delivery of TfRscFv-LipA-antisense HER-2 complex alone or in combination with gemcitabine HCl (GEMZAR®) Gemzar® into nude mice bearing subcutaneous PANC-1 xenograft tumors. The arrow showing the presence of the 17 kDa subunit points to the middle band in the tumor that is not present in either the liver or lung cell samples.

Please replace paragraph [0023], appearing at page 6, with the following amended paragraph:

[0023] Fig. 12 is a graft showing the *in vivo* effect of the combination of TfRscFv-liposome A-antisense HER-2 and gemcitabine HCl (GEMZAR®) Gemzar® treatment on PANC-1 xenograft tumors in comparison to untreated tumors or tumors treated with gemcitabine HCl (GEMZAR®) Gemzar® alone, the complex alone or a combination of gemcitabine HCl (GEMZAR®) Gemzar® and a complex of TfRscFv-liposome A-scrambled HER-2.

Please replace paragraph [0040], appearing at pages 11-12, with the following amended paragraph:

[0040] By measuring the levels of the 17 kDa fragment, one can evaluate and establish the efficacy of a therapy of interest. For example, as described in detail in the examples below, the effects of treatment with a combination of TfRscFv-liposome-AS HER-2 and the chemotherapeutic agent gemcitabine HCl (GEMZAR®) ~~Gemzar® (gemcitabine)~~ on induction of the 17 kDa fragment in mice bearing human pancreatic cancer xenograft tumors who had received multiple i.v. treatments of the antibody fragment-liposome complex carrying either antisense HER-2 or a scrambled HER-2 oligonucleotide plus multiple treatments of gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ were determined. Animals receiving either gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ alone or the antibody fragment-liposome-antisense HER-2 oligonucleotide complex alone were used as controls. Western analysis of serum samples clearly indicated a synergistic induction of the 17 kDa subunit in animals treated with the antisense-containing liposome complex plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ in comparison to treatment with either therapy alone. This strong induction was not evident in mice receiving the scrambled oligo-containing liposome complex plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. This and the other studies described in detail in the Examples demonstrate that the 17 kDa subunit can be used as a non-invasive pharmacodynamic marker for therapeutic efficacy.

Please replace paragraph [0060], appearing at page 14, with the following amended paragraph:

[0060] In one preferred embodiment whole blood was taken from an animal or a human in standard heparinized 3 ml ~~tubes~~ glass specimen tubes (VACUTAINER®) ~~Vacutainer®,~~ CAT#366387, Becton Dickson VACUTAINER® Systems, Franklin Lakes, NJ) containing 45 USP units of Sodium Heparin, mixed well and placed on ice. For small blood volumes 30 µl of 1 x PBS was added to the 3 ml glass specimen tube (VACUTAINER®) ~~(VACUTAINER®) tube~~ to dissolve the Heparin and 1/25 to 1/50 ratio of Heparin/blood volume desired was placed in a sterile microcentrifuge tube. To this tube 50-100 µl of fresh blood was added, mixed well and placed on ice. The blood/Heparin mixture was centrifuged at 1000 x g at 4°C for 10 minutes (large volumes were transferred from the glass specimen tubes (VACUTAINER®) ~~(VACUTAINER®) tube~~ to a sterile microcentrifuge tube prior to

centrifugation). After centrifugation the plasma was removed and placed into a separate sterile microcentrifuge tube. The plasma could be aliquoted and frozen at -70° - -80°C.

Please replace paragraph [0061], appearing at pages 14-15, with the following amended paragraph:

[0061] In another preferred embodiment, blood was collected in heparinized tubes and plasma obtained as above. To remove other whole blood components that might interfere with detection of the 17 kDa fraction the plasma could be purified using the commercially available MICRO BIO-SPIN® ~~“Micro Bio-Spin”®~~ Chromotography Columns (Bio-Rad Laboratories, Hercules CA). Either the P6 column (in Tris) or the P30 column (in Tris) could be used. However, in the preferred embodiment P6 (in Tris) was used. The plasma was purified according to the manufacturer’s protocol except that in one embodiment before Step 2 (centrifuging the column to remove the remaining packing buffer) the column was washed once by gravity with 1 ml of 10 mM Tris-HCl buffer pH=7.4-8.0 without sodium azide. The 17 kDa protein was in the flow through. Figure 1 shows the 17 kDa cleaved caspase 3 fragment purified in this manner from P30 and P6 columns. The positive control was unpurified mouse plasma spiked with protein lysate from PANC-1 cells treated *in vitro* with gemcitabine HCl (GEMZAR®) ~~gemcitabine~~ which induces apoptosis. The negative controls were void volume proteins, mainly albumin, from a P30 column using gravity flow rather than centrifugation.

Please replace paragraph [0084], appearing at page 22, with the following amended paragraph:

[0084] Also evaluated *in vitro* was the induction of the 17 kDa fragment by the combination of the TfRscFv-LipA-AS HER-2 plus the chemotherapeutic agent gemcitabine HCl (GEMZAR®) ~~Gemzar® (gemcitabine)~~. As shown in Figure 7, treatment (as above) of PANC-1 cells for 9 hours with 0.8 µM gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ did not result in the expression of this fragment above background levels. In contrast, treatment with TfRscFv-LipA-AS HER-2 (at 1 µM ODN) plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ (0.8 µM) induced a strong 17 kDa band which was not present in cells treated with

the complex carrying the same amount of SC ODN in combination with the same amount of gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. This indicates that this was not a non-specific ODN or gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ effect. Actin levels showed equal protein loaded per lane.

Please replace paragraph [0085], appearing at page 22, with the following amended paragraph:

[0085] The 17 kDa protein also can be used as a non-invasive *in vivo* pharmacodynamic marker for establishing the efficacy of AS HER-2 therapy. The effects of combination treatment (TfRscFv-LipA-AS HER-2 plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~) on induction of the 17 kDa fragment in mice bearing PANC-1 xenograft tumors that had received multiple (a total of 19) i.v. treatments of TfRscFv-LipA, carrying either AS HER-2 or SC ODN (9 mg/kg) plus 11 i.p. injections of gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ (60 mg/kg) were determined. Animals receiving either gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ or the TfRscFv-LipA AS HER-2 complex alone were used as controls.

Please replace paragraph [0086], appearing at pages 22-23, with the following amended paragraph:

[0086] Plasma was isolated from 1 ml of whole blood from each animal as described above in Example 2. 30 µl of each plasma sample were run on a 4-20% gradient polyacrylamide/SDS gel. The 17 kDa cleaved active subunit of caspase 3 was identified by Western analysis as described in Examples 3 and 4. Western analysis of plasma samples clearly indicated a synergistic induction of the 17 kDa fragment in animals treated with TfRscFv-LipA AS HER-2 plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ compared to treatment with either therapy by itself (Figure 8). This strong induction was not evident in the mice receiving SC ODN (TfRscFv-LipA-SC ODN) plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. There thus is a clear correlation between treatment and effect of either wtp53 or AS HER-2 and the presence of this marker of apoptosis. These studies demonstrate that this protein can be used as a non-invasive pharmacodynamic marker for therapeutic efficacy.

Please replace paragraph [0087], appearing at pages 23-24, with the following amended paragraph:

[0087] Treatment of Pancreatic Cancer (PanCa) with the tumor targeting TfRscFv-LipA-AS HER-2 complex can down-regulate HER-2 expression (even when not overexpressed), thus negatively affecting cell growth/survival and positively enhancing apoptotic pathways leading to increased tumor cell killing. To demonstrate that HER-2 down-regulation via the TfRscFv-liposome complex can affect down-stream cell signaling pathways the ability of this complex to affect components of the PI3K/AKT pathway and apoptosis in PanCa cell lines PANC-1 and COLO357 was assessed by Western analysis. These two cell lines were chosen because they have different levels of HER-2 expression; COLO357 expresses significantly higher HER-2 levels than PANC-1. The phosphorothioate sequence specific AS HER-2, complementary to the initiation codon region (5'-TCC ATG GTG CTC ACT-3'), and the control, non-sequence specific SC (5'-CTA GCC ATG CTT GTC-3') ODNs were synthesized and purified by reverse phase HPLC by Ransom Hill Biosciences (Ramona, CA). Screening of both the AS and SC sequences against the GenBank Database indicated that the AS ODN had homology only to HER-2, while there was no homology between the SC ODN and any sequence in the database. PANC-1 or COLO 357 cells were seeded/in a six well plate and transfected 24 hours later with the TfRscFv-LipA complex carrying 1 μ M (for PANC-1) or 0.5 μ M (for COLO 357) AS HER-2 or SC HER-2 ODN (negative control). The cells were transfected with either oligo alone or, to look for a synergistic effect, in combination with gemcitabine HCl (GEMZAR®) ~~gemicitabine (Gemzar®)~~. At the indicated times, the cells were harvested, lysed in RIPA buffer, protein determined, run (60 ug total protein/lane) on a 4-20% gradient polyacrylamide/SDS gel and transferred to nitrocellulose for Western analysis as described in Examples 1, 3 and 4. To detect HER-protein expression the membranes were probed with the anti-human HER-2/Neu (C-18) rabbit polyclonal Ab (Santa Cruz Biotechnology) and the signal detected by ECL (Amersham). Change in protein expression as compared to untreated cells was also ascertained for total and/or phosphorylated Akt (Ser 473), a central component in the PI3K pathway (using an anti-Human polyclonal Ab, Cell Signaling

Technology), phosphorylated BAD (Ser 136), an important factor in regulation of apoptosis (using an anti-human rabbit polyclonal antibody, Cell Signaling Technology), as well as cleaved caspase 3 (Asp 175) (using the rabbit polyclonal antibody, specific for the 17 kDa subunit, Cell Signaling Technology) and PARP/cleaved PARP (poly ADP ribopolymerase, another marker of apoptosis) using an anti-human rabbit polyclonal antibody, (Cell Signaling Technology) both downstream indicators of apoptosis.

Please replace paragraph [0088], appearing at page 24, with the following amended paragraph:

[0088] Figures 9A and 9B show the effect of transfection of TfRscFv-LipA-AS HER-2, alone or in combination with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~, eight hours post-transfection. The half-life of the HER-2 protein has been reported to be between 10 and 25 hours (Bae et.al *Experimental and Molecular Medicine* 33:15-19 (2001)). Thus, as expected, no changes in HER-2 protein levels by Western analysis were detected at this early time. However, this time point was chosen in an effort to detect early antisense specific effects or any synergistic effect of the combination of AS HER-2 plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. In the PANC-1 cells (Fig 10A), there was some effect on the phosphorylated, active form of AKT by the combination of AS HER-2 and gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. However, a clear synergistic down-modulation by AS HER-2 plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ was evident on the expression of pBAD even at this early time. More significantly, the cleaved forms of caspase 3 (appearance of the 17kDa protein) and PARP, both indicative of the induction of apoptosis, appeared only in the cells treated with the AS HER-2 ODN, primarily in the combination therapy (but faintly with the single therapy as well) and not in those cells treated with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ alone or with SC ODN plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~, indicating that these effects are AS HER-2 specific. COLO357 cells were also examined for changes in protein expression 8 hrs post-transfection. As observed with PANC-1, at this point in time there was virtually no change in HER-2 expression and only minimal down modulation of pAKT. However, here also both cleaved caspase 3 (17kDa subunit) and cleaved PARP are clearly evident in both the cells treated with AS HER-2 alone and in combination with

gemcitabine HCl (GEMZAR®) ~~Gemzar®~~. This, taken together with the fact that there is little or no evidence of these bands in the cells treated with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ only or the combination of SC ODN plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~, again demonstrate that this is an AS specific effect.

Please replace paragraph [0089], appearing at page 25, with the following amended paragraph:

[0089] Since at 8 hours the phosphorylated active form of AKT (pAKT) showed only minimal effect of TfRscFv-LipA-AS HER-2 treatment, PANC-1 cells also were examined 16 hours post-transfection (Figure 10). As this time point was still less than the reported half-life of the HER-2 protein, there was no HER-2 down-modulation evident, as expected. However, significant down modulation of pAKT was observed in the cells treated with AS HER-2, both alone and in combination with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~, that was not evident in the controls. pBAD is even further down-modulated at 16 hrs as compared to 8 hrs, its protein expression almost totally eliminated. A gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ effect also now was observed. The cleaved forms of caspase 3 (17 kDa fragment) and PARP were evident not only in the antisense treated cells but also in those treated with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ alone or with SC ODN plus drug. However, with respect to PARP, there was still a significant difference between the cells treated with AS HER-2 ODN and the controls. In both AS HER-2 single and combination treatment the overall level of PARP (cleaved and uncleaved) was much less than that observed with gemcitabine HCl (GEMZAR®) ~~Gemzar®~~ or SC ODN plus gemcitabine HCl (GEMZAR®) ~~Gemzar®~~, presumably due to earlier onset and continued degradation as a result of AS HER-2 treatment. It should also be noted that for both AKT and BAD the inactive, unphosphorylated forms of these proteins were unaffected by AS HER-2 treatment, supporting the idea that the observed down-modulation is pathway specific and not a result of general non-specific cytotoxicity of the treatment.

Please replace paragraph [0091], appearing at pages 25-26, with the following amended paragraph:

[0091] PANC-1 tumors were induced by implantation of $<1\text{mm}^3$ tumor sections from serially passaged PANC-1 xenograft tumors into 4-6 week old female nude mice. When the tumors reached approximately $100\text{-}200\text{ mm}^3$ the TfRscFv-LipA-AS HER-2 complex was i.v. injected into the tail vein daily for six days. The dose of ODN (AS or SC) administered per mouse was $10\text{ mg/kg/injection}$. For comparison to standard therapy, a separate animal received chemotherapeutic agent gemcitabine HCl (GEMZAR®) Gemzar® (i.p.) only ($60\text{ mg/kg/injection}$) every other day to a total of three injections. In addition, one mouse received the combination of TfRscFv-LipA-AS HER-2 and gemcitabine HCl (GEMZAR®) Gemzar® at the above dose and schedule, and, as a control, one received the combination of complex carrying SC ODN and gemcitabine HCl (GEMZAR®) Gemzar® at the above dose and schedule. All mice were sacrificed 24 hours after the last injection and tumor, liver and lung were harvested as in Example 1. To assess tumor specific targeting in this model HER-2 and the 17 kDa cleaved caspase 3 fragment expression in the tissues was examined by Western Blot analysis Examples 3 and 4. The effect of TfRscFv-LipA-AS HER-2 on induction of the 17kDa fragment in tumor and tissue samples also screened for HER-2 levels was examined. Western analysis as described in Examples 3 and 4, clearly shows induction of the 17 kDa fragment in the tumor from animals treated with TfRscFv-LipA AS HER-2 alone or plus gemcitabine HCl (GEMZAR®) Gemzar® (Figure 11). This strong induction was not evident in the mice receiving SC ODN (TfRscFv-LipA-SC ODN) plus gemcitabine HCl (GEMZAR®) Gemzar® or gemcitabine HCl (GEMZAR®) Gemzar® alone. More importantly, this 17kDa cleaved caspase 3 band was not evident in any of the liver or lung samples. These studies demonstrate that after intravenous administration, the complex could preferentially target and deliver the AS HER-2 ODN to tumor. In addition, the expression of the 17 kDa was evident only where the therapeutic molecule was expressed.

Please replace paragraph [0092], appearing at pages 26-27, with the following amended paragraph:

[0092] The *in vitro* studies described above indicated that treatment of PanCa cells with the TfRscFv-LipA-AS HER-2 complex could increase their response to gemcitabine

HCl (GEMZAR®) Gemzar®. For this gene therapy delivery system to be clinically relevant for human cancers, e.g., PanCa, the increased sensitization observed *in vitro* must translate to an *in vivo* model. The efficacy of the TfRscFv-LipA-AS HER-2 in treating PanCa *in vivo* was assessed using the subcutaneous PANC-1 xenograft mouse model. Athymic nude mice (5-9 mice/group with two tumors/mouse) bearing subcutaneous xenograft tumors of <50 mm³ were treated three times per week with the TfRscFv-LipA-AS HER-2 complex containing ODN at 9mg/kg/injection. As controls one group of animals received gemcitabine HCl (GEMZAR®) Gemzar® alone, the TfRscFv-LipA-AS HER-2 alone, or the combination of gemcitabine HCl (GEMZAR®) Gemzar® and the complex carrying the SC ODN. gemcitabine HCl (GEMZAR®) Gemzar® was given I.P. twice weekly at 60 mg/kg. The animals received a total of 18 i.v. injections of complex and 12 of gemcitabine HCl (GEMZAR®) Gemzar®. As shown in Figure 12, gemcitabine HCl (GEMZAR®) Gemzar® alone had only minimal effect on tumor growth, while AS HER-2 only was ineffective. The groups receiving gemcitabine HCl (GEMZAR®) Gemzar® alone or control SC ODN plus gemcitabine HCl (GEMZAR®) Gemzar® are not statistically different, indicating that any growth inhibition by TfRscFv-LipA-SC ODN plus Gemzar® is strictly a drug effect. However, tumor growth was substantially inhibited in the mice that received the combination of TfRscFv-LipA-AS HER-2 and gemcitabine HCl (GEMZAR®) Gemzar®. The differences between the group receiving the combination therapy and gemcitabine HCl (GEMZAR®) Gemzar® alone or TfRscFv-LipA-AS HER-2 alone are highly statistically significant ($p < 0.001$ by student's t-test). Thus, i.v. administration of the complex carrying AS HER-2, in combination with gemcitabine HCl (GEMZAR®) Gemzar®, is efficacious against PanCa.

Please replace paragraph [0097], appearing at pages 29-30, with the following amended paragraph:

[0097] To establish that the results observed in the animal model can be applied to humans and that the expression of the 17 kDa cleaved caspase 3 fragment can be used to non-invasively assess therapeutic effect, a matched set of serum samples was obtained from two human patients who had been treated for breast cancer using conventional chemotherapy.

These serum samples were obtained before (pre-) and after (post-) treatment. The serum was purified using the P₆ (in Tris) MICRO BIO-SPIN® ~~Micro Bio-Spin®~~ Chromatography Columns. (Bio-Rad Laboratories, Hercules, CA)(Example 2). The flow-through from the columns was diluted at a ratio of serum to RIPA buffer of from 0.1:1, to 10:1, preferably at 1:1. Equal volumes (1 to 100 µl) were run on a 4-20% polyacrylamide/SDS gel, transferred and probed for expression of the 17 kDa cleaved caspase 3 fragment as described in Examples 3 and 4. As shown in Figure 14, the 17 kDa cleaved caspase 3 fragment is not evident in the serum from either a control (non-cancer bearing) human subject or the patients pre-treatment. This band is clearly present, however, post-standard chemotherapy. Thus, as shown in the animal model, the expression of the 17 kDa cleaved caspase 3 fragment, an indicator of apoptosis, does correlate with cancer therapies (gene, antisense, and chemotherapy) in human patients. Thus, for any therapy that induces apoptosis, including radiation therapy, analysis of whole blood (as serum or plasma) for the 17 kDa cleaved caspase 3 fragment, as described in the Examples contained in this application, can be a relatively non-invasive method to monitor the effectiveness of the therapy. In human cancer patients it is envisioned that whole blood (1 ml to 3 ml) can be drawn in heparinized tubes and centrifuged at 300 to 1000 x g, at 4° to 27°C for 3 to 10 minutes to obtain plasma. This plasma can be run directly (as described in Examples 3 and 4) or further purified by centrifugation of a 20-75 µl aliquot of the sample through a P6 or P30 MICRO BIO-SPIN® ~~Micro Bio-Spin®~~ Chromatography Column (preferably P6) at 300 to 2000 x g (preferably 1000 x g) for 1 to 10 minutes (preferably 4 minutes) at 4° to 27°C (preferably 18-24°C, most preferably 20°C). The flow-through is diluted with RIPA buffer at a ratio of plasma to RIPA of 0.1:1 to 10:1, preferably 1:1 before electrophoresis on a 4-20% polyacrylamide/SDS gel, transferred to any nylon or nitrocellulose solid support membrane, preferably PROTRAN® ~~Protran®~~ (S+S), with a pore size of 0.1 to 0.45 µm, preferably 0.22 µm. Detection is performed using a polyclonal or monoclonal anti-caspase 3 antibody that detects the 17 kDa fragment, preferably only the 17 kDa fragment, by radioactive or non-radioactive means, preferably non-radioactive, preferably non-colorimetric, preferably via chemiluminescence, preferably enhanced chemiluminescence such as found in the ECL Western Blotting detection reagents and analysis system (Amersham Biosciences, Piscataway, NJ), with

exposure to autoradiography film including, but not limited to Hyperfilm ECL, for times ranging from 30 seconds to 24 hours, preferably 1 minute to 18 hours.